

In Vivo Observations of Larval Brooding in the Chilean Oyster, *Ostrea chilensis* Philippi, 1845*

O. R. CHAPARRO¹, R. J. THOMPSON², AND J. E. WARD³

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland A1C 5S7, Canada

Abstract. The Chilean oyster *Ostrea chilensis* broods its larvae within the mantle cavity. *In vivo* observations, made with an endoscope, have demonstrated that the female does not undergo gill modification before or during the brooding process. The veliger larvae move freely, have no physical connections with the tissues of the mother, and are often seen as an aggregation ("swarm") around the labial palps. The larvae show a distinct circulation pattern inside the mantle cavity of the mother. Sporadic but strong pallial currents eject larvae from the swarm and transport them between the demibranchs to the posterior region, from which some return anteriorly in the basal ciliated tracts of the gills, and others on the marginal ciliated grooves. Larvae frequently move from the basal to the marginal groove of a demibranch via the ordinary filaments, although they occasionally travel in the opposite direction, moving along the principal filaments from the marginal groove to the basal tract. The circulation of the larvae in the mantle cavity may serve to irrigate them, thereby facilitating gas exchange and suspension-feeding.

The veligers are not lecithotrophic, but readily remove and ingest particles suspended in the mantle cavity. The impact on the nutrition of the mother is not yet known, although the presence of particles in the food grooves and tracts and the transfer of material to the palps suggest that the feeding mechanism functions normally during brooding.

Introduction

Brooding of larvae is a mode of reproduction which has evolved in many species within a variety of higher taxa (Webber, 1977; Mackie, 1984; Brahmachary, 1989), and brooding mechanisms vary considerably. Among bivalve mollusks that brood, both freshwater and marine, the larvae (embryos) are contained in the mantle cavity of the female, either in the suprabranchial or the infra-branchial chamber, but always in association with the gill. The larvae are sometimes retained in the interlamellar spaces of both demibranchs or of the inner or outer demibranchs only; alternatively, they may be confined to brood sacs, marsupia, mucous masses, or other specialized structures (Ockelmann, 1964; Solis, 1967; Franz, 1973; Mackie *et al.*, 1974; Heard, 1977; Mackie, 1984; Tankersley and Dimock, 1992, 1993). Most authors have concluded that when the larvae are brooded in specialized structures, they are restricted to those structures (Morton, 1977a; Kabat, 1985; Asson-Batres, 1988; Richard *et al.*, 1991; Tankersley and Dimock, 1992) and show little or no motility until the release period (Mackie, 1984). Several species possess brood masses, often confined by a membrane, and in others the embryos are attached to the demibranchs with byssal threads or affixed to gill papillae (Heard, 1977; Bartlett, 1979; Richardson, 1979; Kabat, 1985; Asson-Batres, 1988; Russell and Huelsenbeck, 1989). Conversely, in species that brood the larvae between the demibranchs, the larvae have been presumed to move within the mantle cavity of the mother, unattached to the maternal tissues. In some cases brooding is sequential, *i.e.*, not all eggs are fertilized at the same time, and embryos are gradually displaced ventrally in the mantle cavity as they mature (Kabat, 1985; Russell and Huelsenbeck, 1989).

The family Ostreidae is probably the most well-known marine bivalve group in which brooding behavior occurs.

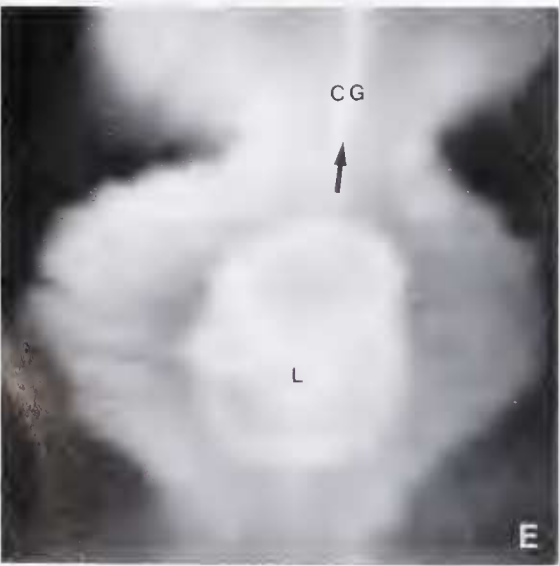
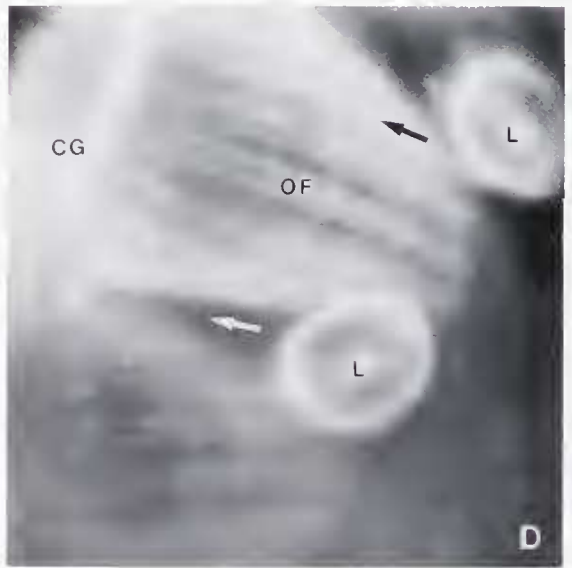
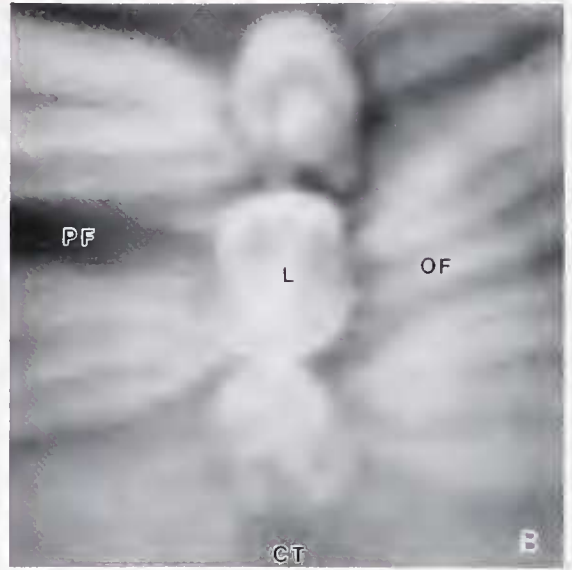
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* A condensed, narrated version of the video sequences is available by sending a blank VHS tape to RJT (format available is NTSC only).

¹ Present address: Instituto de Biología Marina, Universidad Austral de Chile, Casilla 567, Valdivia, Chile.

² Author for correspondence.

³ Present address: Marine Research Group, Department of Biology, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada.



All species of the genus *Ostrea* brood their young (Millar and Hollis, 1963; Galtsoff, 1964; Chanley and Dinamani, 1980; Fernandez Castro and Le Pennec, 1988). Moreover, the brooding takes place within the mantle cavity of the female (*i.e.*, not associated with specialized structures), and the circulation of late blastulae within the cavity has been briefly described in the crested oyster, a member of the closely related genus *Ostreola* (Nelson, 1946). Although the number of larvae produced has also been estimated for several species of brooding oysters (*e.g.*, Winter *et al.*, 1984), very little is known about the brooding process, excepting a limited amount of information for the Chilean oyster *Ostrea chilensis*, which broods the larvae for a longer time (8 weeks) than any other oyster, and releases them at a more advanced stage of development, when they are very large (>450 μm diameter; Toro and Chaparro, 1990). These features make the Chilean oyster particularly suitable for the endoscopic studies we describe in this paper. The pediveligers settle within a few hours of being released by the mother. There is some evidence that the larvae lie on the gill of the mother or in the mantle cavity (Walne, 1963; Solis, 1967; Tomicic and Bariles, 1981; DiSalvo *et al.*, 1983; L pez, 1983), and that the loss in meat content by adult female oysters during spring and summer may be interpreted as a cost of the brooding process (Solis, 1967; Winter *et al.*, 1983).

The nature of the mother-larva relationship in brooding ostreids and the physiology of the larvae and the mother during brooding are not well understood. Among the questions that remain to be addressed are the following. How are the larvae distributed within the mantle cavity? Is there any physical connection between mother and larvae, or do the larvae swim freely inside the mantle cavity of the mother? What is the mechanism for ventilating the larvae? Is the mother able to filter while brooding? Do the larvae interfere with the feeding activity of the mother, as suggested for *Ostrea chilensis* by Winter *et al.* (1983), either physically or by competing with her for suspended particles? If the larvae do feed, what is the mechanism? In this study we addressed these questions by using an endoscope to observe living larvae of *O. chilensis* in the mantle cavity of the mother.

Materials and Methods

Brooding females (48–60 mm long) of *Ostrea chilensis*⁴, an economically important species found only on the south-central coast of Chile, were obtained from the Quempill n Estuary, Chilo  Island (41  52'S; 73  46'W). They were transported on ice to the Marine Sciences Research Laboratory, Memorial University of Newfoundland, where they were held in natural seawater at 17 C and 27‰ S until they were examined with the endoscope. The oysters were fed daily with a variety of cultured algae. Although several of the specimens released some larvae on arrival at the laboratory, most females retained their broods without apparent ill-effects.

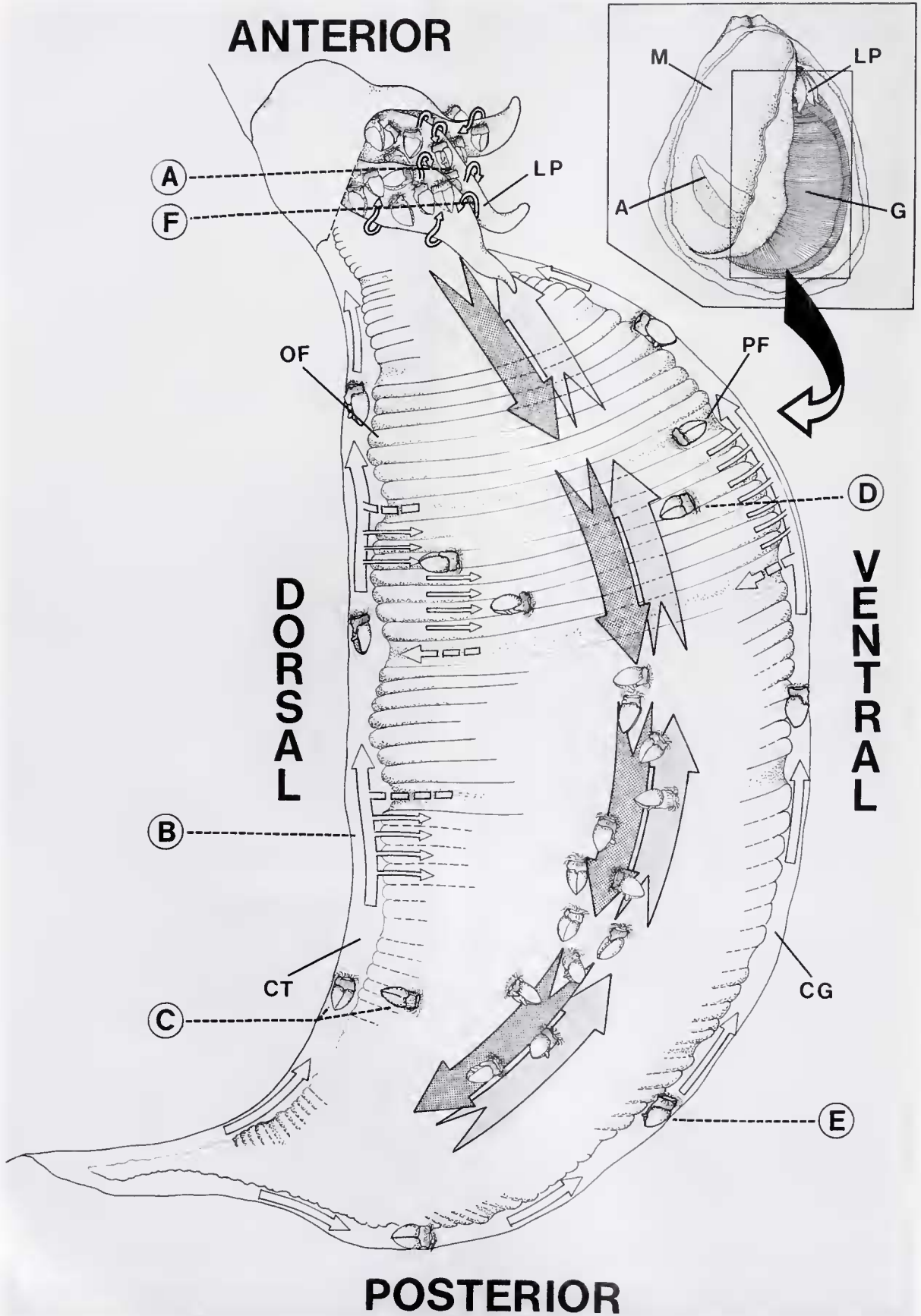
Details of the endoscope methods have been published previously (Ward *et al.*, 1991). The apparatus consisted of an endoscope (Olympus SES 1711D or Scholly 241810.045) mounted on a micromanipulator to facilitate location of the optical insertion tube (OIT; 1.7 mm diameter) within the mantle cavity of the oyster, a cold light source connected to the endoscope with a fiber-optic cable, and a CCD camera (monochrome or color; Cohu 6500 or 8210) coupled to a Hi-8 video recording system (Sony EV-S2000). For some observations, an accessory mirror was fitted to the Scholly endoscope, so that objects at 90  to the OIT could be observed. To permit insertion of the OIT into the mantle cavity, a piece of shell was trimmed from the ventral margin, care being taken to avoid damage to the mantle or other soft tissues.

Most observations were made on the eyed stage of the pediveliger (*ca.* 400 μm diameter), although some were made on trochophores. To determine whether the larvae were capable of feeding, and whether the feeding tracts of the brooding female were able to function, nontoxic, light-reflective red plastic particles (2–10 μm diameter) were introduced through a Pasteur pipette in the vicinity of the inhalent margin of the adult. These particles were clearly visible with the monochrome and color cameras.

⁴ Assigned to the genus *Tiostrea* as *Tiostrea chilensis* by Chanley and Dinamani (1980) and subsequently reassigned to the genus *Ostrea* as *Ostrea (Eostrea) pulchana* by Harry (1985).

Figure 1. Endoscope micrographs of veliger larvae in the pallial cavity of a brooding female oyster (*Ostrea chilensis*). The diameter of each larva is about 400 μm . (A) Swarm of larvae around the labial palps of the mother. (B) Movement of larvae anteriorly within the basal tract. (C, D) Larvae leaving the basal tract and being carried along the ordinary filaments towards the marginal groove of the demibranch. (E) A larva being transported anteriorly along the margin of the demibranch. (F) Larvae returning to the swarm between the labial palps. These micrographs are represented diagrammatically in Figure 2.

L = larva; CT = ciliated tract (basal tract); CG = ciliated groove (marginal groove); OF = ordinary filament; PF = principal filament; LP = labial palp. Arrows indicate direction of movement of larvae.



To determine whether brooding oysters can distinguish between their own larvae and other particles of similar size (biogenic and nonbiogenic), we introduced into the mantle cavity Sephadex beads (175–350 μm diameter) or eggs of the limpet *Crepidula dilatata* (150–300 μm diameter). The oysters were then examined periodically with the endoscope for about 24 h to establish the fate of these particles. Feces and pseudofeces from each oyster were also examined.

Results and Discussion

No physical connections between mother and larvae were detected, and the gill of the mother did not appear to be modified for brooding, nor was there any evidence of special structures in which larvae could be held. Likewise, the larvae were not adapted for adhesion to any part of the adult. Rather, they were observed free in the interdemibranchial spaces and between and around the labial palps, as well as being transported on the ciliary tracts of the gill. These observations are consistent with those of Kabat (1985), who found no anatomical modifications of the gill in *Transennella tantilla* (Veneridae), and suggested that the larvae are not attached to the mother's gills.

The larvae of *O. chilensis* were always located within the infrabranchial chamber, never the suprabranchial, and were prevented from leaving the mantle cavity only by the interdigitating processes of the mantle margin. In other brooding species of bivalve mollusks, individual larvae or small groups are often enveloped by a membrane, which may prevent the premature release of the larvae from the mantle cavity (Bartlett, 1979; Richardson, 1979).

In our study of brooding *O. chilensis*, a large aggregation of larvae (which we term a "swarm") was always observed closely associated with the palps (Fig. 1A, F; Fig. 2A, F). According to Hopkins (1936), the larvae of *O. lurida* also lie adjacent to the labial palps and at the anterior margin of the gill. In the Chilean oyster, we noted that the tips of the palps appeared to manipulate the larvae, keeping the swarm constantly in motion. This palp action may serve to remove mucus, which appears to build up on the larvae as a result of their close proximity to the gill, especially the food grooves, and may also facilitate irrigation of the larvae in a region where they are concentrated.

The larvae circulated within the mantle cavity of the mother in a clearly defined pattern (Figs. 2, 3). At irregular intervals, some of the larvae close to the palps were ejected from the swarm and moved posteriorly by a strong water current produced intermittently by the mother, as described by Nelson (1946) for blastulae (92–105 μm diameter) of the crested oyster *Ostreola equestris*. There was also a weaker counter-current which transported suspended larvae anteriorly. The resultant force was in a posterior direction; the overall impression was of a "sloshing" motion, alternating between the strong posteriorly directed current and the weaker anteriorly directed current. The larvae returned to the palp region via the basal ciliary tracts and the marginal grooves, where they were commonly seen aligned one behind the other (Fig. 1B, E; Fig. 2B, E).

The highest larval fluxes were observed in the basal tract. Some of the larvae were carried from the basal tract to the marginal groove via the frontal tracts of the ordinary filaments (Fig. 1C, D; Fig. 2C, D). In such instances, each larva usually lay within a single plical fold, and contacted the frontal cilia on the ordinary filaments of the two adjacent plicae, which transported it ventrally; on reaching the gill margin, the larva was directed anteriorly towards the palps, presumably by cilia on the crests of the groove (Fig. 1E; Fig. 2E). Occasionally, a larva was observed moving on the frontal surface of the gill along the principal filament of a plical groove, towards the basal tract. The mean velocity of the larvae was 471 $\mu\text{m s}^{-1}$ in the basal tracts and 141 $\mu\text{m s}^{-1}$ in the marginal grooves (measurements made at 17°C). These values are consistent with those obtained at 11–13°C for food particles (approximately 5 μm diameter) transported by the same means in the eastern oyster *Crassostrea virginica*, viz. 413–697 $\mu\text{m s}^{-1}$ in the basal groove and 101–193 $\mu\text{m s}^{-1}$ in the marginal groove (Ward *et al.*, 1993). Thus the brooded larvae of *O. chilensis* are moved anteriorly in the basal tracts and marginal grooves by the same mechanism as the food particles, which are much smaller. Furthermore, the aggregation of larvae around the labial palps of the mother is maintained, in part, through the transportation of larvae by ciliary action on the gill, a mechanism which was not apparent to Hopkins (1936) when he observed

Figure 2. Schematic lateral view of a single demibranch and the labial palp region of *Ostrea chilensis*, showing the circulation pattern followed by the larvae in a brooding female. The orientation follows the convention that the buccal region is anterior. To be consistent with the terminology followed by most students of ostreids, the text refers to the basal tract rather than the dorsal groove, and to the marginal groove rather than the ventral groove. Circled letters correspond to the endoscope micrographs in Figure 1. Broad arrows show the direction in which larvae are transported by currents in the mantle cavity (dark arrow = strong flow producing resultant movement in a posterior direction; light arrow = weaker counter-flow in an anterior direction, producing a "sloshing" action). Thin arrows show the routes taken by larvae that are in contact with the gill; broken arrows indicate that larval transport is intermittent.

A = adductor muscle; G = gill; LP = labial palps; M = mantle; CT = ciliated tract (basal tract); CG = ciliated groove (marginal groove); OF = ordinary filament; PF = principal filament.

concentrations of larvae around the palps of brooding *O. lurida*.

While returning from the posterior region of the mantle cavity to the anterior region, the larvae were constantly in motion, but normally remained in contact with the gill. Larvae located within the swarm, or moving posteriorly in the mantle current, rotated and oscillated continuously, using their vela. Circulation of the larvae through the mantle cavity, however, was achieved by the currents generated by the mother, because larval transport in a posterior direction ceased when the mother stopped pumping, even though the velar ciliary tracts remained active. Furthermore, movement in an anterior direction was not influenced by the orientation of the active velum.

Because their eggs are relatively large, oysters of the genus *Ostrea* are often believed to be lecithotrophic (Mackie, 1984). Gallardo (1989) considered the larvae of *O. chilensis* to be lecithotrophic, a view supported by the observation of DiSalvo *et al.* (1983) that they do not ingest microalgae when reared outside the mantle cavity of the mother. Our *in vivo* endoscopic examination of larvae of the Chilean oyster, however, showed that red plastic particles drawn into the mantle cavity by the mother were captured by the vela and were visible in the stomachs of the larvae. Mucus strings containing red particles were occasionally seen adhering to the larvae, but we could not determine whether the larvae were ingesting these mucus-bound particles. The larva, at least during the brooding pediveliger stage, is clearly capable of ingesting suspended particles, which is consistent with the suggestion by Solis (1973) that the larva is planktotrophic during the short pelagic phase.

We conclude that the brooded larvae obtain food indirectly from the mother by removing particles suspended in the mantle cavity and possibly by ingesting the mucus string from the food grooves. Such modes of feeding have not been reported previously for bivalve species, although Buroker (1985) has suggested that brooded oyster veligers (*Ostrea* spp.) can feed in the ventilatory current of the mother. Mackie (1979) has postulated a similar feeding mechanism for the larvae of some freshwater bivalves (Pisidiidae), in which the mother removes particles from the incoming water current, and the extramarsupial larvae may secondarily filter the water that has passed through the gill ostia of the mother.

Some bivalve species seem to transfer nutrients directly from the mother to the larvae during the brooding period. For example, larvae may obtain nutrients from maternal tissues (Purchon, 1968), from mucus secreted by cells in the interlamellar septa (Wood, 1974), from secretions of the hypobranchial gland (Morton, 1978), from epithelial secretions of the interfilamentar and interlamellar junctions (Morton, 1977b), or from branchial papillae to which the embryos adhere (Bartlett, 1979). Furthermore, ele-

ments such as calcium may be transferred to embryos maintained in brood chambers (Tankersley and Dimock, 1992).

According to Solis (1967), the meat content of Chilean oysters decreases during winter, spring, and summer. The weight losses in winter and spring can be attributed to low food availability and to spawning, respectively, but during summer spawning is complete and food availability is high. The loss of body weight in summer occurs during the brooding period, and has been attributed to a reduced feeding capability resulting from the presence of the larval brood (Winter *et al.*, 1983). The proposed mechanism was physical interference by the larvae in the normal functioning of the gill, rather than competition for food between the mother and the larvae. Our observations with the endoscope showed clearly that the larvae were removing particles from suspension in the mantle cavity of the brooding female, and that there was no apparent interference with the feeding process of the mother. Thus the larvae were concentrated around the palps and in the basal tracts and marginal food grooves of the gill, yet particle retention by the gill filaments appeared to be unimpeded. Furthermore, the particles intercepted by the gill moved to the basal and marginal tracts in the usual way (Ward *et al.*, 1993), and mucus-bound particle strings were observed being transported from the marginal grooves to the labial palps, despite the presence of larvae in these channels. In fact, larvae were often observed adhering to the mucus string and being carried across the marginal gill-palp junction to the palps themselves. Whether feeding by the larvae significantly diminishes the food available to the mother, thereby reducing energy input, and whether the presence of the brood, especially the swarm around the palps, may reduce the efficiency with which the brooding female can process food particles, are possibilities that remain to be tested.

Higher energy costs may also contribute to weight loss during the brooding period. During brooding, the clearance rate of the female may increase to compensate for the retention of particles by the larvae; there would be an energy cost associated with such a response. There may also be a significant cost to the mother in generating the currents that transport the larvae through the pallial cavity. Some studies have considered the energy costs associated with brooding in marine invertebrates. For example, brooding sea stars continually manipulate and aerate the young, and the energy allocated to reproduction may therefore be partitioned between gonad development and brood handling (Menge, 1974, 1975). Brooding octopods expel water from the mantle cavity more frequently than nonbrooding individuals, because the former irrigate their egg masses (Brahmachary, 1989); presumably additional energy costs are incurred through this behavior.

Brooding Chilean oysters distinguished between their own larvae and large foreign particles introduced into the

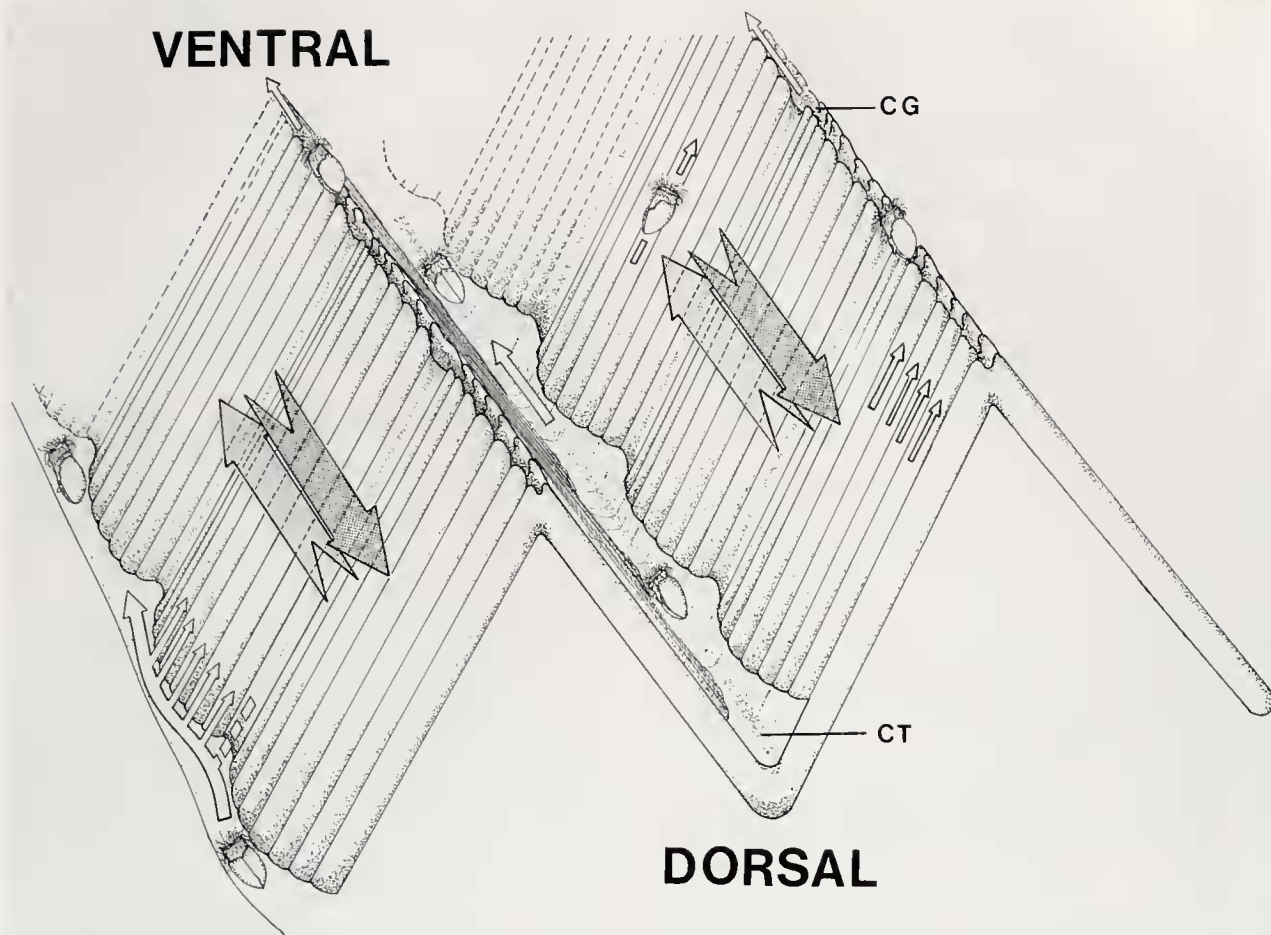


Figure 3. Schematic representation of two adjacent demibranchs in *Ostrea chilensis* (posterior view), showing the circulation of larvae between them. Symbols as in Figure 2.

mantle cavity, whether the latter were biogenic (eggs of *Crepidula dilatata*, 150–300 μm diameter) or nonbiogenic (Sephadex beads 175–350 μm diameter). Most of the Sephadex beads were eliminated as pseudofeces by the mother, but some were detected in the feces. Discrimination between Sephadex beads and larvae did not appear to be perfect, however, because intact larvae and fragments of larvae were sometimes found in the feces alongside Sephadex particles, although these ingested larvae may have been individuals that the mother identified as non-viable. *Crepidula* eggs were observed in the pseudofeces of the mother, but never in the feces, perhaps because they were completely digestible.

This paper is the first attempt to understand the brooding process in the Chilean oyster. We have shown that the veliger larvae can move freely in the mantle cavity of the mother, exhibiting a well-defined circulation pattern that may serve to facilitate gas exchange and perhaps to maximize contact with suspended particles, because the veligers are able to feed. We have preliminary observations suggesting that trochophore larvae are brooded in a similar manner to veligers. Nevertheless, some questions remain

unanswered. What is the impact of the brooding process on the physiological condition of the mother? In particular, is the mother's food intake significantly reduced during brooding, and what proportion of the food collected by the mother is ingested by the larvae? These and related issues are currently being addressed as we assess the advantages and disadvantages of brooding as a mode of reproduction in this species.

Acknowledgments

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